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The Use of Piperonyl Butoxide and MGK-264 to Improve the Efficacy of Some Plant-Derived Molluscicides

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Abstract: Synergism of an oil of *Azadirachta indica*, a powdered extract of *Allium sativum* bulbs and an oleoresin of *Zingiber officinale* rhizomes by piperonyl butoxide and MGK-264 was studied against the snails *Lymnaea acuminata* and *Indoplanorbis exustus*. The active components of these plant-derived molluscicides, respectively azadirachtin, allicin and [6]gingerol, were also combined with these synergists. Both piperonyl butoxide and MGK-264 enhanced the toxicity of all of the test compounds. The response of snails to the synergised mixtures was both time- and dose-dependent. © 1998 Society of Chemical Industry

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Key words: synergist; piperonyl butoxide; MGK-264; molluscicide; snail

1 INTRODUCTION

The snails *Lymnaea acuminata* and *Indoplanorbis exustus* are the intermediate hosts of the liver flukes *Fasciola hepatica* and *F. gigantica*. These flukes are the causative agent of endemic fascioliasis in the northern part of India. Synthetic pesticides alone and with different synergists have been used against both these snails,[1–3] and several plant-product molluscicides have also been identified for their control.[4] It has been observed that neem-oil (*Azadirachta indica* A. Juss), a powdered extract of *Allium sativum* L. (garlic) bulbs and an oleoresin of *Zingiber officinale* Roscoe (ginger) rhizome are potent molluscicides.[5–7] In the present study the synergists piperonyl butoxide and MGK-264 have been used with these three plant-derived molluscicides and with their active components azadirachtin, allicin and [6]-gingerol against the snails *L. acuminata* and *I. exustus*.

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2 MATERIALS AND METHODS

Azadirachta indica oil was supplied by Indian Herbs, Saharanpur; powdered *Allium sativum* bulb and the oleoresin were prepared using the methods of Singh and Singh and Singh *et al.*[5,7] Allicin was prepared from alliin by the method of Mohammad and Woodward.[8] [6]-Gingerol was supplied by Tsumura and Co., Japan and azadirachtin by T. Stains & Co. Ltd, India. *Azadirachta indica* oil and *Zingiber* oleoresin along with piperonyl butoxide and MGK-264 were used with non-ionic emulsifier shnehakshar (Indian Herbs, Saharanpur).

Adult *L. acuminata* and *I. exustus* ($2{\cdot}25(\pm 0{\cdot}20)$ and $0{\cdot}85(\pm 0{\cdot}037)$ cm in length) were collected locally, acclimatised to laboratory conditions for 72 h and used as test animals. Toxicity experiments followed the method of Singh and Agarwal.[1] Ten experimental animals were kept in each glass aquarium containing 3 litres dechlorinated tap water. The snails were exposed for 96 h to different concentrations of plant-derived molluscicides and molluscicide + synergists piperonyl butoxide (α[2-(2-butoxyethoxy)ethoxy]-4,5-methylenedioxy-2-propyltoluene; McLaughlin Gormley King Co. USA) or MGK-264 (*N*-octyl bicycloheptenedicarboximide;

TABLE 1
Toxic Effect of *Azadirachta indica* Oil with Synergists MGK-264 (MGK) or Piperonyl Butoxide (PB) (1 : 5) against *Lymnaea acuminata* and *Indoplanorbis exustus* at Different Exposure Periods

Period (*h*)	*Treatment*[a]	L. acuminata *toxicity* LC_{50} (*LCL-UCL*) (*mg litre*$^{-1}$)	*Synergistic ratio*[b]	*Slope* (±*SD*)	I. exustus *toxicity* LC_{50} (*LCL-UCL*) (*mg litre*$^{-1}$)	*Synergistic ratio*[b]	*Slope* (±*SD*)
24	*A. indica* oil	17·35 (14·58–23·58)		2·75 (±0·51)	8·80 (7·49–10.91)		2·37 (±0·44)
	A. indica oil + PB	8·82 (8·09–9·83)	1·96	4·53 (±0·72)	3·50 (2·71–6·18)	2·52	1·87 (±0·39)
	A. indica oil + MGK	6·84 (6·12–7·87)	2·53	3·56 (±0·54)	3·23 (2·43–6·31)	2·72	1·78 (±0·47)
48	*A. indica* oil	13·47 (11·70–16·18)		2·90 (±0·48)	4·48 (3·77–5·04)		4·06 (±0·63)
	A. indica oil + PB	6·89 (6·22–7·50)	1·94	4·62 (±0·70)	2·12 (1·66–3·12)	2·11	1·52 (±0·37)
	A. indica oil + MGK	5·32 (4·66–6·02)	2·52	3·19 (±0·50)	1·86 (1·54-2·39)	2·40	1·99 (±0·42)
72	*A. indica* oil	8·99 (7·00–1·84)		2·02 (±0·46)	2·19 (1·67-2·69)		2·09 (±0·26)
	A. indica oil + PB	4·92 (4·31–5·51)	1·80	3·45 (±0·50)	1·44 (1·08-1·80)	1·52	1·69 (±0·37)
	A. indica oil + MGK	3·98 (3·23–4·58)	2·25	2·96 (±0·50)	1·18 (0·73-1·49)	1·85	1·63 (±0·41)
96	*A. indica* oil	5·76 (4·26–6·85)		3·01 (±0·53)	1·26 (0·99-1·56)		2·00 (±0·25)
	A. indica oil + PB	1·60 (0·47–2·54)	3·60	0·83 (±0·25)	0·96 (0·66-1·18)	1·31	2·10 (±0·41)
	A. indica oil + MGK	1·89 (1·28–2·46)	3·00	1·53 (±0·27)	0·83 (0·52-1·04)	1·51	2·18 (±0·46)

[a] Six batches of 10 snails were exposed to different concentrations of *A. indica* oil + PB/MGK combinations in 1 : 5 ratio. Mortality was determined every 24 h. Concentrations given are the final concentration in the aquarium water.
[b] Synergistic ratio (LC_{50} of molluscicide/ LC_{50} of molluscicide + synergist PB/MGK).

McLaughlin Gormley King Co. USA) mixed in 1 + 5 ratio.

Six aquaria were used for each concentration and control animals were held in similar conditions. Mortality was recorded at 24-h intervals up to 96 h exposure periods. Dead animals were removed at each observation to avoid contamination of aquarium water. No response to a needle probe was taken as evidence of death.

Lethal concentration (LC_{50}) values, lower and upper confidence limits (LCL and UCL) and slope values were calculated according to the method of Russell *et al.*[9]

3 RESULTS

Combinations of *A. indica* oil, *A. sativum* powder and oleoresin of *Z. officinale* and their pure compounds, azadirachtin, allicin and [6]-gingerol with the synergists

TABLE 2
Toxic Effect of *Allium sativum* Powder with Synergists MGK-264 (MGK) or Piperonyl Butoxide (PB) (1 : 5) against *Lymnaea acuminata* and *Indoplanorbis exustus* at Different Exposure Periods

Period (*h*)	*Treatment*[a]	L. acuminata *toxicity* LC_{50} (*LCL-UCL*) (*mg litre*$^{-1}$)	*synergistic ratio*[b]	*slope* (±*SD*)	I. exustus *toxicity* LC_{50} (*LCL-UCL*) (*mg litre*$^{-1}$)	*synergistic ratio*[b]	*slope* (± *SD*)
24	Powder alone	903 (669–833)		2·13 (±0·09)	85·7 (74·5–103·2)		2·99 (±0·44)
	Powder + PB	2·84 (2·24–3·88)	318·05	1·66 (±0·27)	24·0 (18·6–34·3)	3·56	1·51 (±0·28)
	Powder + MGK	2·26 (1·77–3·01)	399·55	1·56 (±0·27)	24·0 (18·6–34·3)	3·56	1·51 (±0·28)
48	Powder alone	522 (435–703)		2·23 (±0·44)	64·4 (55·4–74·7)		2·61 (±0·41)
	Powder + PB	1·54 (1·19–19·3)	339·18	1·72 (±0·27)	15·90 (11·82–21·15)	4·04	1·40 (±0·27)
	Powder + MGK	1·09 (0·80–1·35)	479·21	1·39 (±0·30)	16·5 (11·9–22·9)	3·90	1·26 (±0·26)
72	Powder alone	374 (308–460)		2·05 (±0·43)	50·3 (43·6–57·0)		3·22 (±0·43)
	Powder + PB	0·81 (0·52–1·06)	471·12	1·77 (±0·31)	7·61 (4·29–10·51)	6·60	1·32 (±0·26)
	Powder + MGK	0·73 (0·54–0·88)	511·65	2·24 (±0·43)	7·54 (4·52–10·22)	6·66	1·44 (±0·27)
96	Powder alone	271 (230–307)		3·21 (±0·49)	39·2 (31·7–45·6)		2·58 (±0·44)
	Powder + PB	0·72 (0·48–0·90)	376·47	1·81 (±0·39)	3·32 (0·97–5·57)	11·81	1·26 (±0·29)
	Powder + MGK	0·62 (0·54–0·69)	473·19	3·93 (±0·74)	3·46 (1·16–5·64)	11·33	1·33 (±0·29)

[a] Six batches of 10 snails were exposed to different concentrations of *A. sativum* powder + PB/MGK combination in 1 : 5 ratio. Mortality was determined every 24 h. Concentrations given are the final concentration in the aquarium water.
[a] Synergistic ratio (LC_{50} of molluscicide/LC_{50} of molluscicide + synergist PB/MGK).

TABLE 3
Toxic Effect of Oleoresin of *Zingiber officinale* with Synergists MGK-264 (MGK) or Piperonyl Butoxide (PB) (1 : 5) against *Lymnaea acuminata* and *Indoplanorbis exustus* at Different Exposure Periods

Period (h)	*Treatment*[a]	L. acuminata *toxicity* LC_{50} *(LCL–UCL) (mg litre*$^{-1}$*)*	*Synergistic ratio*[b]	*Slope* ($\pm$*SD*)	I. exustus *toxicity* LC_{50} *(LCL–UCL) (mg litre*$^{-1}$*)*	*Synergistic ratio*[b]	*Slope* ($\pm$*SD*)
24	Oleoresin alone	No mortality			No mortality		
	Oleoresin + PB	4·94 (3·58–6·01)		1·69 (±0·29)	80·3 (62·1–132·0)		2·03 (±0·40)
	Oleoresin + MGK	1·98 (1·47–2·72)		1·33 (±0·26)	50·2 (33·1–139·8)		1·10 (±0·28)
48	Oleoresin alone	64·8 (47·5–176·9)		1·94 (±0·54)	296·6 (245·2–454·3)		3·06 (±0·68)
	Oleoresin + PB	1·90 (1·35–2·42)	34·65	1·70 (±0·27)	39·1 (32·4–48·0)	7·57	2·08 (±0·35)
	Oleoresin + MGK	1·12 (0·78–1·43)	58·78	1·63 (±0·28)	18·6 (14·5–23·5)	15·96	1·68 (±0·28)
72	Oleoresin alone	47·1 (27·3–82·9)		1·93 (±0·49)	189·7 (167·4–212·9)		3·53 (±0·66)
	Oleoresin + PB	1·11 (0·76–1·46)	42·47	1·43 (±0·25)	19·1 (13·2–23·9)	9·94	1·99 (±0·35)
	Oleoresin + MGK	0·77 (0·60–0·91)	61·23	2·43 (±0·44)	9·39 (6·44–12·00)	20·20	1·84 (±0·30)
96	Oleoresin alone	29·1 (24·3–35·5)		2·20 (±0·48)	148·6 (127·8–167·7)		3·14 (±0·58)
	Oleoresin + PB	0·65 (0·39–0·88)	44·83	1·67 (±0·29)	7·57 (4·95–10·06)	19·62	1·60 (±0·25)
	Oleoresin + MGK	0·61 (0·53–0·68)	47·77	4·25 (±0·75)	3·95 (2·73–5·09)	37·68	1·87 (±0·27)

[a] Six batches of 10 snails were exposed to different concentrations of oleoresin of *Z. officinale* + PB/MGK combination in 1 : 5 ratio. Mortality was determined every 24 h. Concentrations given are the final concentration in the aquarium water.
[b] Synergistic ratio (LC_{50} of molluscicide/LC_{50} of molluscicide + synergist PB/MGK).

piperonyl butoxide or MGK-264 showed that the molluscicidal activity of these mixtures was time- and dose-dependent against both species of snails. There was a significant negative correlation between the exposure time and LC_{50} of the mixtures (Tables 1–4). Mixtures of *A. indica* oil + piperonyl butoxide or MGK-264 against *L. acuminata* and *I. exustus* were more toxic than *A. indica* oil alone (Table 1). The synergistic ratios of *A. indica* oil + piperonyl butoxide mixture against *L. acuminata* and *I. exustus* were, at any exposure period, more than 1·8 and 1·3, respectively. The synergistic ratios of *A. indica* oil + MGK-264 against both the snails were generally, but not always, significantly, more pronounced at any exposure period (more than 2·25 and 1·51) than *A. indica* oil + piperonyl butoxide mixture (Table 1).

Molluscicidal activity of *A. sativum* powder against *L. acuminata* was synergised more than 318 times when mixed with either piperonyl butoxide or MGK-264 (Table 2). Molluscicidal activity of this combination against *I. exustus* was only 3 to 11 times higher than that of *A. sativum* powder (Table 2).

Mixtures of oleoresin of *Z. officinale* with either piperonyl butoxide or MGK-264 were synergised more than 34 and 47 times, respectively, against *L. acuminata* (Table 3) and 7 and 15 times, respectively, against *I. exustus* than the oleoresin alone (Table 3). The order of synergism of piperonyl butoxide and MGK-264 against the snail *L. acuminata* was *A. sativum* powder > *Zingiber* oleoresin > *A. indica* oil, whereas in the case of *I. exustus*, the order of synergism was *Zingiber* oleoresin > *A. sativum* powder > *A. indica* oil.

Combinations of the active compounds of *A. indica* oil, *A. sativum* powder and oleoresin of *Z. officinale* with piperonyl butoxide or MGK-264 (Table 4) showed that the molluscicidal activity of azadirachtin was increased 3 to 11 times by piperonyl butoxide and 5 to 35 times by MGK-264. Molluscicidal activity of allicin was increased 5 to 9 times by piperonyl butoxide and 6 to 16 times by MGK-264. Molluscicidal activity of [6]-gingerol was increased 8 to 11 times by piperonyl butoxide and 7 to 19 times by MGK-264.

4 DISCUSSION

It is evident from the results that piperonyl butoxide and MGK-264 synergise the molluscicidal activity of *A. indica* oil, *A. sativum* powder and oleoresin of *Z. officinale*. Piperonyl butoxide and MGK-264 alone are not toxic to either species.[3,10] Piperonyl butoxide and MGK-264 are commonly used with carbamates, organophosphates and pyrethroids for the control of different pests.[1,3,10–12] Piperonyl butoxide and MGK-264 usually exert their synergistic action with synthetic pesticides by inhibiting the mixed function oxidase activity which detoxifies xenobiotics[11,13] or they may increase the penetration of the toxin which results in a high titer of toxin at the active site. In the present study, synergistic action of these two synergists with *A. indica* oil, *A. sativum* bulb powder and oleoresin of *Z. officinale* may be due to the inhibition of microsomal oxidases which reduces the detoxification of the chemical components present in the plant extract. Singh *et al.*[6] and Singh and Singh[14] reported that the main components which cause snail death in *A. indica* oil are

TABLE 4
Toxic Effects of Azadirachtin, Allicin and [6]-Gingerol with Synergists MGK-264 (MGK) or Piperonyl Butoxide (PB) (1 : 5) against *Lymnaea acuminata*

Period (*h*)	*Treatment*[a]	*Toxicity* LC_{50} (*LCL-UCL*) (*mg litre*$^{-1}$)	*Synergistic ratio*[b]	*slope* ($\pm SD$)
24	Azadirachtin	0·35 (0·28–0·47)		1·82 (±0·31)
	Azadirachtin + PB	0·09 (0·07–0·13)	3·88	2·72 (±0·54)
	Azadirachtin + MGK	0·07 (0·06–0·09)	5·00	2·18 (±0·37)
	Allicin	6·34 (5·74–8·50)		2·31 (±0·48)
	Allicin + PB	1·22 (1·08–1·54)	5·19	4·12 (±0·74)
	Allicin + MGK	1·02 (0·83–1·54)	6·21	2·62 (±0·55)
	[6]-Gingerol	7·23 (5·26–13·41)		1·39 (±0·30)
	[6]-Gingerol + PB	0·87 (0·72–1·30)	8·31	2·21 (±0·50)
	[6]-Gingerol + MGK	0·94 (0·67–1·82)	7·69	1·69 (±0·35)
48	Azadirachtin	0·35 (0·28–0·47)		1·82 (±0·31)
	Azadirachtin + PB	0·07 (0·06–0·08)	5·00	2·80 (±0·50)
	Azadirachtin + MGK	0·04 (0·03–0·05)	8·75	1·83 (±0·29)
	Allicin	5·45 (4·59–6·40)		2·46 (±0·48)
	Allicin + PB	0·83 (0·74–0·93)	6·56	3·64 (±0·62)
	Allicin + MGK	0·52 (0·45–0·60)	10·48	2·98 (±0·49)
	[6]-Gingerol	5·42 (3·98–9·04)		1·26 (±0·27)
	[6]-Gingerol + PB	0·55 (0·47–0·64)	9·85	2·67 (±0·48)
	[6]-Gingerol + MGK	0·56 (0·42–0·89)	9·67	1·40 (±0·28)
72	Azadirachtin	0·35 (0·28–0·47)		1·82 (±0·31)
	Azadirachtin + PB	0·05 (0·04–0·05)	7·00	2·65 (±0·48)
	Azadirachtin + MGK	0·02 (0·017–0·02)	17·15	2·06 (±0·28)
	Allicin	4·48 (3·53–5·28)		2·32 (±0·47)
	Allicin + PB	0·56 (0·50–0·61)	8·00	5·60 (±0·79)
	Allicin + MGK	0·37 (0·31–0·42)	12·10	3·39 (±0·52)
	[6]-Gingerol	3·64 (2·46–5·79)		1·02 (±0·26)
	[6]-Gingerol + PB	0·33 (0·25–0·45)	11·03	1·40 (±0·27)
	[6]-Gingerol + MGK	0·19 (0·15–0·24)	19·15	2·05 (±0·28)
96	Azadirachtin	0·35 (0·28–0·47)		1·82 (±0·31)
	Azadirachtin + PB	0·03 (0·02–0·03)	11·66	1·69 (±0·27)
	Azadirachtin + MGK	0·01 (0·01–0·02)	35·00	2·02 (±0·29)
	Allicin	3·64 (2·65–4·34)		2·61 (±0·57)
	Allicin + PB	0·40 (0·35–0·45)	9·10	3·81 (±0·53)
	Allicin + MGK	0·22 (0·16–0·27)	16·54	1·91 (±0·28)
	[6]-Gingerol	1·87 (1·31–2·40)		1·64 (±0·27)
	[6]-Gingerol + PB	0·19 (0·14–0·24)	9·84	1·98 (±0·28)
	[6]-Gingerol + MGK	0·12 (0·09–0·15)	15·58	1·91 (±0·26)

[a] Six batches of 10 snails were exposed to different concentrations of pure compound azadirachtin; allicin and [6]-Gingerol + PB/MGK combination in 1 : 5 ratio. Mortality was determined every 24 h. Concentrations given are the final concentration in the aquarium water.
[b] Synergistic ratio (LC_{50} of molluscicide/LC_{50} of molluscicide + synergist PB/MGK).

azadirachtin, terpenoids and sulfur compounds. It seems that the use of piperonyl butoxide or MGK-264 reduces the detoxification of these compounds resulting in higher toxicity. Lange[15] and Lange and Feuerhake[16] have reported that piperonyl butoxide caused 50–80% increase in the effectiveness of different extracts of neem products against 4th-instar larvae of *Plutella xylostella* (L.) and *Leptinotorsa decemlineata* Say.

The synergistic action of piperonyl butoxide and MGK-264 with powder of *A. sativum* bulb was very high, reducing LC_{50} values by over 300 times, which is higher than recorded for any synthetic pesticide combination with these two synergists, e.g. deltamethrin 3·73 and 18·66 times against both the snails.[3,17] To have maximum effect, the synergist must penetrate the organism and be transported to the active site rapidly. The higher level of synergism in snails may be due to more rapid penetration of the synergist through the soft foot of the snails than through insect cuticle. The main component responsible for snail death in *A. sativum*

powder is allicin,[5,18] which causes a significant inhibition of acetylcholinesterase, lactic dehydrogenase and alkaline phosphatase activity in the nervous tissue of *Lymnaea acuminata*.[19] A high titer of allicin in the snail's body may be maintained by these synergists, either by inhibiting its detoxification or by increasing the penetration of allicin. Synergistic action of piperonyl butoxide and MGK-264 with oleoresin was also very effective, perhaps due to the inhibition of detoxification of the active component, [6]-gingerol.[7]

In conclusion, combinations of the synergists piperonyl butoxide and MGK-264 with plant-derived mollusicides are very effective. These synergists can potentiate the efficacy and reduce the dosage of plant-derived molluscicides, so that areas of treated water where the concentrations of toxins are sub-lethal may still exert a lethal effect by synergism.

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